THE USE OF THE RABBIT IN EXPERIMENTAL CHEMOTHERAPY OF TUBERCULOSIS*

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While streptomycin therapy has been eminently successful in certain types of human tuberculosis, the results have been far short of those anticipated on the basis of studies in animals. This is not altogether surprising, since the effect of the antibiotic has been tested in guinea pigs and mice. These were subjected to a rapidly progressive disease which spreads in the body chiefly by focalization of small numbers of microorganisms *via* hematogenous and lymphogenous routes. To determine whether a given therapeutic agent is likely to be effective against chronic ulcerative phthisis in man, where the bacilli multiply inordinately in the septic membrane of the walls of cavities, and where dissemination takes place chiefly by bronchogenic spread of large numbers of tubercle bacilli, it is obvious that the agent should be assayed against a disease in animals which closely simulates the human infection.

Chronic ulcerative pulmonary phthisis, with the essential pathogenetic characteristics of the human disease, can be produced with regularity in rabbits when they are exposed to the inhalation of known numbers of virulent bovine tubercle bacilli.†

Materials and Methods

An apparatus has been constructed at the Henry Phipps Institute for quantitative airborne infection of rabbits or other laboratory animals, modeled after the one elaborated by Wells[1] and modified in certain essential respects to improve its quantitative aspects and increase the safety of its operation. FIGURE 1 is a schematic drawing of the instrument.

Briefly, a fine suspension of almost completely isolated, virulent, bovine type tubercle bacilli is freed from clumps by brisk centrifugation and filtration through a medium sintered glass filter. The suspension is then atomized through a nozzle by compressed air in a specially designed flask. The large droplets settle at once on the walls of the flask. The droplet nuclei, which are visible only by the Tyndal effect, are carried through a long pipe into a chamber in which the rabbits are exposed. Only the head of the animal protrudes into the chamber. The rest of the rabbit's body is enclosed in a cylinder separated from the chamber by an iris diaphragm which fits comfortably but snugly about the neck of the animal. The concentration of the tubercle-bacilli particles in the air respired by the rabbits is determined by a Wells air centrifuge or a Rosebury[2] impinging sampler provided with a calibrated inclined draft gauge which records the volume of air sampled. In these two air-sampling devices, the bacteria in the air are

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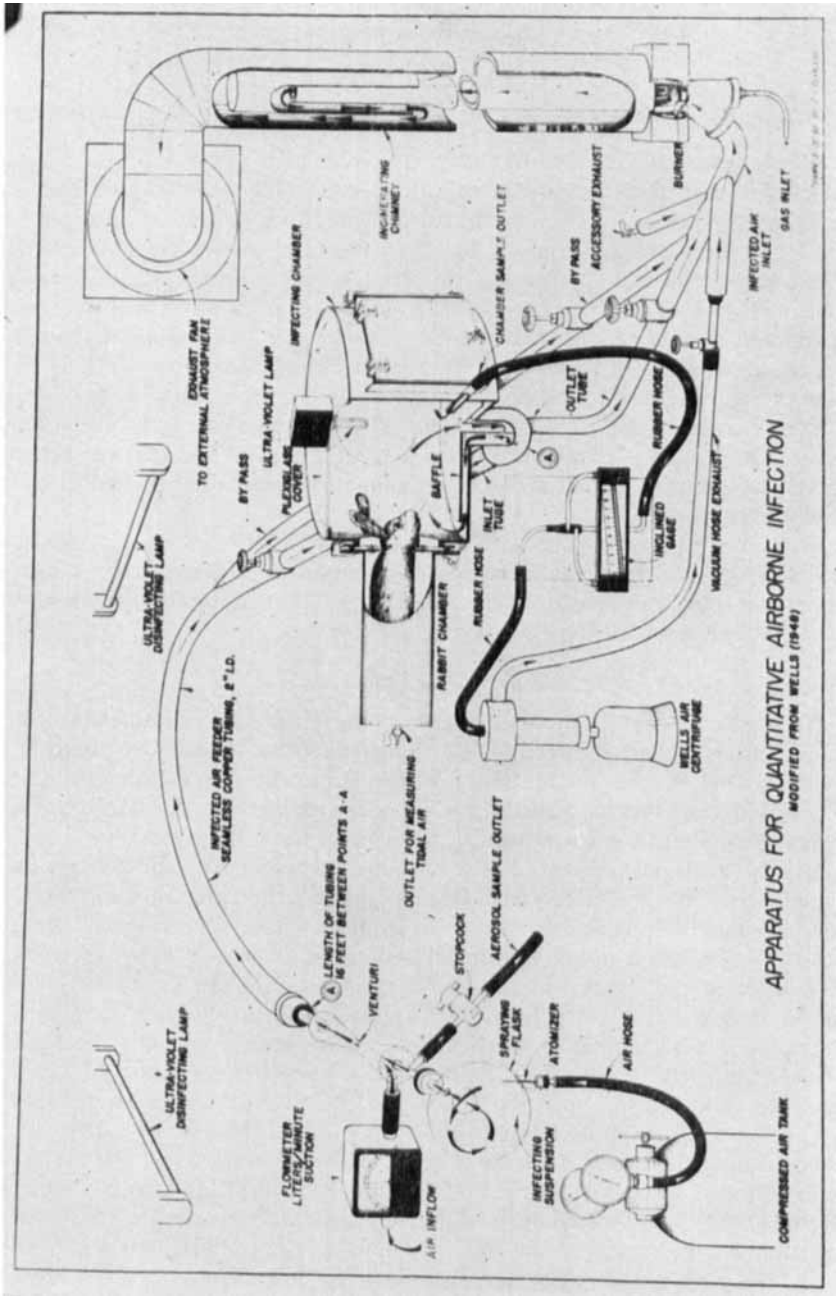

APPARATUS FOR QUANTITATIVE AIRBORNE INFECTION
MODIFIED FROM WELLS (1948)
ULTRA-VIOLET DISINFECTING LAMP
INFECTED AIR FEEDER SEAMLESS COPPER TUBING, 2" I.D.
LENGTH OF TUBING 16 FEET BETWEEN POINTS A-A
VENTURI
FLOWMETER LITERS PER MINUTE SUCTION
AIR INFLOW
STOPCOCK
OUTLET FOR MEASURING TIDAL AIR
AEROSOL SAMPLE OUTLET
SPRAYING FLASK
ATOMIZER
AIR HOSE
INFECTING SUSPENSION
COMPRESSED AIR TANK
ULTRA-VIOLET DISINFECTING LAMP
EXHAUST FAN TO EXTERNAL ATMOSPHERE
BY PASS
ULTRA-VIOLET LAMP
INFECTING CHAMBER
PLEXIGLAS COVER
BAFFLE
INLET TUBE
RABBIT CHAMBER
RUBBER HOSE
INFECTING CHAMBER
CHAMBER SAMPLE OUTLET
BY PASS
ACCESSORY EXHAUST
BURNER
OUTLET TUBE
RUBBER HOSE
INCLINED GAGE
VACUUM HOSE EXHAUST
WELLS AIR CENTRIFUGE
INFECTED AIR INLET
GAS INLET

FIGURE 1

TABLE 1

RATIO BETWEEN THE NUMBER OF TUBERCLE BACILLI ESTIMATED AS INHALED AND THE NUMBER CULTURED FROM THE LUNGS

Date of experiment	*Strain used*	*Rabbit numbers*	*Number of bacilli estimated as inhaled*	*Number of bacilli cultured directly (Dir.) or after treatment(Tr.)*	*Percentage of bacilli estimated as inhaled actually recovered from lungs*
Oct. 15, '47	Rav. S	A 10-77	2646	907 (Tr)	34
Feb. 10, '48	"	M-14	194	255 (Tr)	131
	"	M-15	167	317 (Tr)	190
Mar. 8, '48	"	M-29	213	33 (Tr)	16
	"	M-31	157	29 (Tr)	19
May 4, '48	"	A-7	594	287 (Tr)	48
	"	M-56	564	222 (Tr)	39
May 26, '48	"	C-8	843	397 (Dir)	47
May 6, '49	"	III R2-2	1648	2634 (Tr)	160
Nov. 13, '47	Rav. R	I	1425	1898 (Dir)	133
	"	J	1346	565 (Tr)	42
Jan. 7, '48	"	AD 2-21	94	99 (Tr)	101
Jan. 22, '48	"	M-11	138	75 (Tr)	54
Mar. 10, '48	"	FC 1-6	442	720 (Dir)	163
Mar. 29, '48	"	M-37	553	146 (Tr)	26
	"	M-42	572	172 (Dir)	30
Apr. 2, '48	"	M-46	2190	1027 (Dir)	47
Apr. 20, '48	"	M-52	555	130 (Tr)	23
	"	M-54	518	530 (Tr)	102
Dec. 27, '47	H37RV	III 3-1	413	534 (Dir)	129
Nov. 8, '48	"	Ca 4-9	3597	3045 (Dir)	85
Apr. 1, '49	"	C 7-47	192	81 (Tr)	42

TABLE 2

RATIO BETWEEN THE NUMBER OF TUBERCLE BACILLI ESTIMATED AS INHALED AND THE NUMBER CULTURED FROM THE LUNGS

Number of experiments.....22
Average recovery.....76% ± 52
In $\frac{1}{3}$ of experiments from 16 to 40% were recovered
In $\frac{1}{3}$ of experiments from 42 to 100% were recovered
In $\frac{1}{3}$ of experiments from 101 to 190% were recovered

thrown into a known volume of glycerine broth either by centrifugal force or by the impingement of a jet of air at high velocity. This broth is then quantitatively cultured. Knowing the concentration of the tubercle bacilli

in the air respired by the exposed animals and the duration of this exposure, one can calculate the number of bacilli inhaled by the rabbits by using Kleifer's[3] formula, which gives the volume of air an animal must respire in a given time to satisfy its oxygen requirements. The infected air is sucked out of the exposure chamber by an incinerating chimney and is drawn to the outside atmosphere by a fan, after the destruction of the contained bacilli. The entire system is under negative pressure and the worker is further protected by a sufficiency of strategically-located ultraviolet lamps. There is another ultraviolet lamp in the exposure chamber itself which, with the aid of rapid fresh air exchange, helps to remove almost instantaneously any residual organisms from the chamber at the cessation of exposure.

TABLE 3

FATE OF RABBITS OF RACE III SENSITIZED WITH HEAT-KILLED TUBERCLE BACILLI AND EXPOSED 5.5 MONTHS LATER TO THE INHALATION OF ABOUT 50 VIRULENT BOVINE TUBERCLE BACILLI

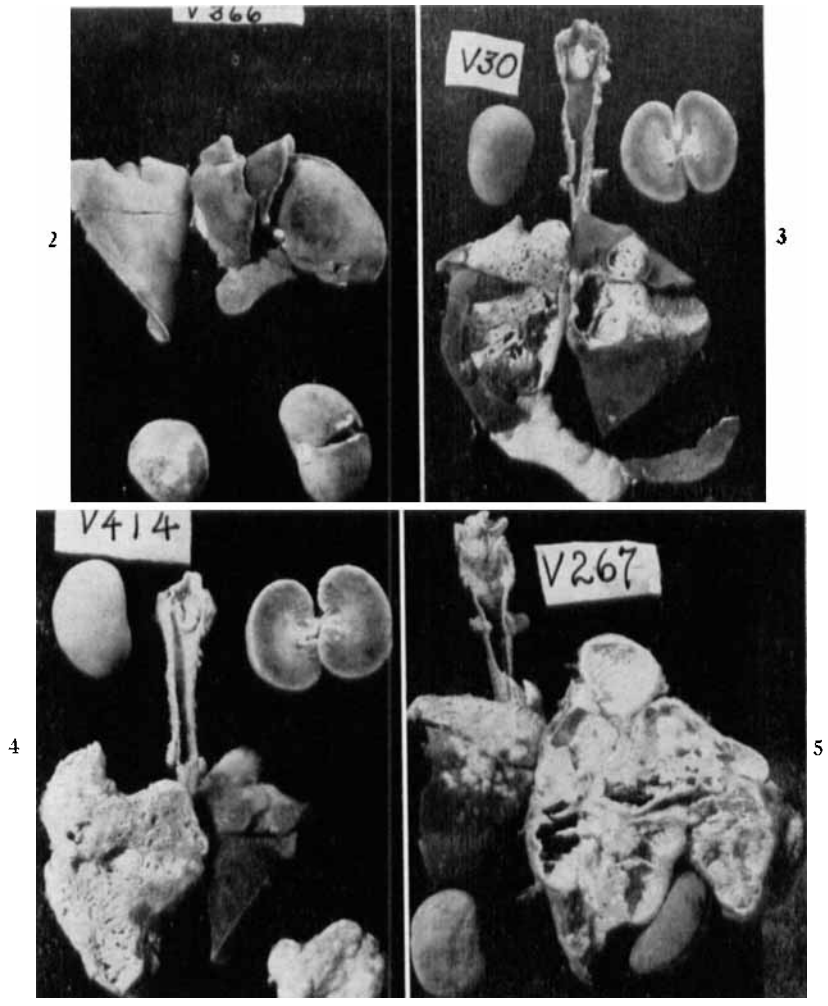
Rabbit number	*Survival in months after exposure*	*Type of tuberculosis*
V 369*	still living, 26	No evidence of tuberculosis
U 784	killed, 4.7	No tuberculosis
V 366	9.5	Three small, well walled-off cavities in right lung. No tuberculosis elsewhere, including hilum nodes. Large infarcts in both kidneys.
V 30	10.1	One large cavity with limited bronchogenic spread in the upper lobe of each lung. Hilum nodes normal. Ulcerative tuberculosis of larynx. Single miliary tubercle in one kidney.
V 414	7.3	Unilateral ulcerative pulmonary tuberculosis with slight, contralateral lesions. Hilum nodes normal. Miliary tubercles in each kidney. Ulcerative laryngeal tuberculosis. One large tuberculous pleural nodule. Tuberculosis of one wrist joint.
V 267	6.6	Completely excavated tuberculosis of all lobes of right lung, including the azygous lobe. Consolidation of upper lobe of left lung and bronchogenic spread to lower lobe of same lung. Hilum nodes—normal. Few miliary tubercules in one kidney.

* This rabbit was not sensitized with heat-killed tubercle bacilli before exposure.

TABLES 1 and 2 present an evaluation of the accuracy of the estimate of the number of bacillary units inhaled by the rabbits. In 22 successive experiments, involving both virulent bovine and human-type tubercle bacilli, where the exposed rabbits were killed immediately after exposure, and where the lungs were quantitatively cultured, it was found that an average of 76 per cent of the bacilli calculated as inhaled were recovered from the lungs. Since, in two thirds of the experiments, from 40 to more than 100 per cent of the calculated number of bacilli were actually cultured from the lungs, it is evident that, while the dosage is not quite accurate, it is reasonably so for this kind of biological experimentation.

Ulcerative Pulmonary Phthisis in Rabbits

Having established in preliminary experiments that, under certain conditions, about 9 ± 7 tubercle-bacilli units were required to generate one



2 3 4 5

FIGURES 2–5

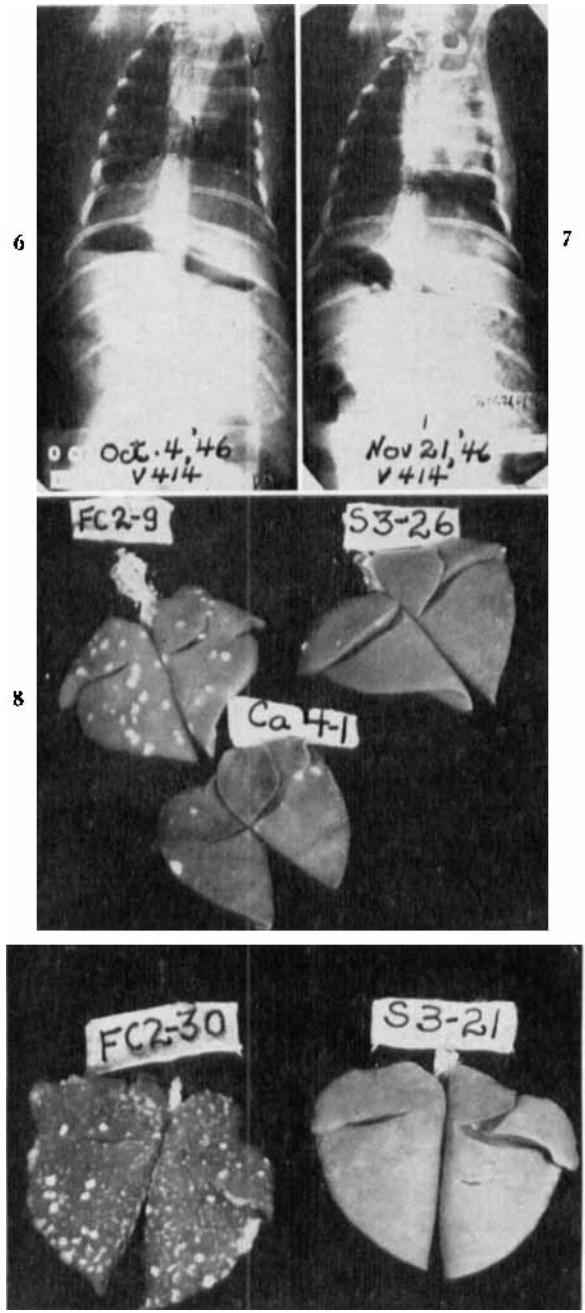
FIGURE 2. The lungs and kidneys of rabbit V 366 at death, 9.5 months after exposure. One of the three primary cavities in the right lung is seen in the lower lobe. Infarcts of nontuberculous origin in both kidneys.

FIGURE 3. The organs of rabbit V 30 at death, 10.1 months after exposure. One large cavity in each lung with limited bronchogenic spread in both. Tracheobronchial nodes and kidneys, normal. Ulcerative tuberculous laryngitis with tubular spread to the appendix, seen below left lung.

FIGURE 4. Organs of rabbit V 414 at death, 7.3 months after exposure. Unilateral ulcerative tuberculosis in left lung with slight bronchogenic dissemination in the upper lobe of right lung. Tracheobronchial nodes and kidneys, normal. Ulcerative tuberculous laryngitis. Tuberculous pleural nodule in the right lower corner of photograph. The progression of the disease in this rabbit during the first 4 months of the infection is depicted in FIGURES 6 and 7.

FIGURE 5. The organs of rabbit V 267 at death, 6.6 months after exposure. Completely excavated tuberculosis of all lobes of right lung, including azygous lobe which is depicted just above the right kidney. Consolidation of upper lobe of left lung and bronchogenic spread to lower lobe of the same lung. Tracheobronchial nodes, normal. The few, minute, miliary tubercles in the kidneys cannot be seen.

tubercle in the lung,[4] a group of inbred rabbits of Race III, obtained from Dr. Paul B. Sawin of the Roscoe B. Jackson Memorial Laboratory, were vaccinated intracutaneously with heat-killed tubercle bacilli. As the re-



FIGURES 6–9 (*For description see facing page*).

sult of preliminary observations, it was found that most of the rabbits of this family were genetically highly resistant to tuberculosis.

About 5 months after vaccination, these rabbits were exposed to the inhalation of about 50 tubercle bacilli of the Ravenel strain, at one sitting. It was found that these rabbits showed all degrees of resistance as seen in man. TABLE 3 and FIGURES 2, 3, 4, and 5 illustrate the varying types of progression of localized ulcerative pulmonary phthisis in these rabbits. These varied from failure of the disease to take root at all, to the formation of primary ulcerated lesions which did not progress beyond their site of inception, and to limited bronchogenic dissemination from the original ulcerated foci, as well as to unilateral ulcerative phthisis. The most extensive and rapidly progressive disease was seen in one rabbit, in which the ulcerative tuberculosis had destroyed one lung completely, leaving nothing but thick-walled cavities from each of the lobes. In the contralateral lung, the disease was progressing from the upper to the lower lobes by bronchogenic spread. There was little or no lymphogenous or hematogenous dissemination from the pulmonary portal of entry in these rabbits, including the tracheal bronchial nodes.[5] The similarity of the disease thus generated under quantitative conditions to the various types of the so-called adult or reinfection type of ulcerative pulmonary pthisis in man is striking.

FIGURES 6 and 7 illustrate the *in vivo* progression of the disease in one of these rabbits. The cavity in the left upper lobe of this rabbit, about 2 months after inhalation, can be clearly visualized in the X-ray photograph of FIGURE 6. The progression of the disease, including that of the cavity, in this rabbit can be seen in FIGURE 7.

It is evident, therefore, that one can produce ulcerative phthisis at will under quantitative conditions of natural inhalation infection. Such a disease would be eminently suitable for the assay of chemotherapeutic agents directed at the elimination of the most prevalent and contagious type of tuberculosis, which thus far has proved generally refractory to streptomycin therapy. The method also affords an opportunity for the study of the effect of therapeutic agents on the progression and healing of cavities, as these can be visualized by serial X ray, in the same manner as is done in man.

Finally, since it has been shown by a number of observers[6] that the emergence of streptomycin-resistant strains is fostered by the inordinate growth of the bacilli in cavities, it is evident that the tuberculous cavities developed by these rabbits exposed to quantitative inhalation of bovine tubercle bacilli

FIGURES 6–9 (*See opposite page*).

FIGURE 6. Radiograph of rabbit V 414, 67 days after exposure. Three foci are visible. A walled-off cavity in the third interspace and areas of consolidation in the sixth and eighth interspaces, respectively, of the left lung, are indicated by arrows.

FIGURE 7. Radiograph of the rabbit shown in FIGURE 2, 48 days later. The progression of the disease in the left lung is evident. The right lung is normal.

FIGURE 8. The lungs of rabbit S3-26, Ca 4-1, and FC 2-9 of high, intermediate and low genetic resistance to tuberculosis, respectively, 32 days after the inhalation of 3000–4000 tubercle bacilli. In the most resistant rabbit, S3-26, 1000 tubercle bacilli generated a single tubercle. In the rabbit of intermediate resistance, Ca 4-1, one tubercle resulted from the inhalation of 140 bacilli. In the rabbit of lowest resistance, FC 2-9, one tubercle resulted from the inhalation of 40 bacilli.

FIGURE 9. The lungs of resistant rabbit S3-21 and of susceptible rabbit FC 2-30 166 to 167 days after the simultaneous inhalation of 2000 human-type tubercle bacilli. The lungs of the susceptible rabbit show numerous tubercles throughout both lungs. The lungs of the resistant rabbit are free of grossly visible tubercles.

may offer a method of studying *in vivo* the emergence of strains of tubercle bacilli resistant to a given therapeutic agent.

Quantitative Inhalation of Human-Type Tubercle Bacilli in Genetically Susceptible Rabbits

Since the above experiments were completed, it has been found that rabbits can also be used for short-term experiments on the *in vivo* bacterici-

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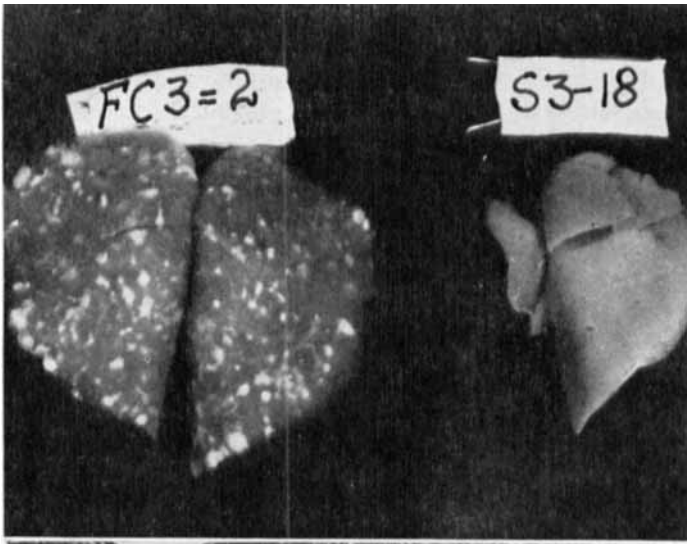

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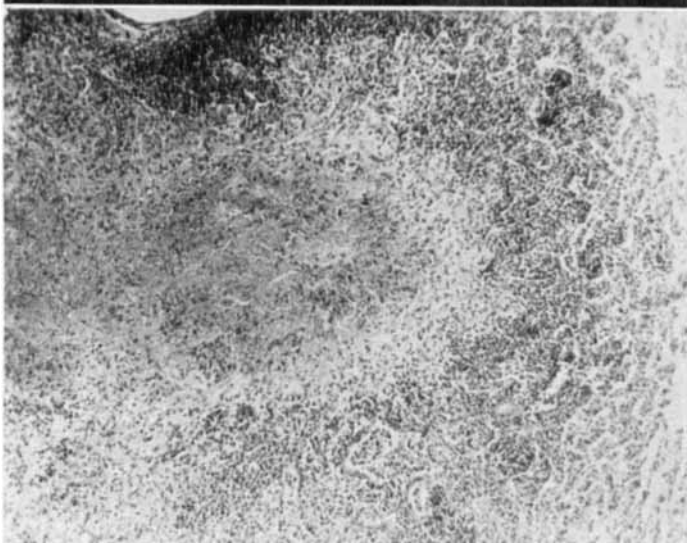

FIGURES 10, 11

FIGURE 10. The lungs of resistant rabbit S3-18 and of susceptible rabbit FC 3-2, 165 to 168 days after a simultaneous exposure to 2000 human-type tubercle bacilli. No tubercle bacilli could be recovered from the left lung of S 3-18 after guinea pig inoculation. The grossly unaffected right lung of S 3-18 is to be contrasted with the extensively diseased lung of FC 3-2.

FIGURE 11. Microphotograph of a lesion in the lung of the susceptible rabbit FC 2-7, 47 days after the inhalation of about 2000 tubercle bacilli of the human type. The typical character of the caseous tuberculous lesion is evident.

dal or bacteriostatic effects of chemotherapeutic agents.[7] If highly inbred rabbits of low genetic resistance to tuberculosis inhale several thousand virulent, human-type tubercle bacilli, their lungs will be seeded with numerous caseous tubercles some 30 days after inhalation. When rabbits of highly genetic resistance are simultaneously and quantitatively exposed, few or no primary foci develop. In fact, the number of tubercles generated in response to the inhalation of a certain number of human-type tubercle bacilli is a function of the native resistance of the given rabbits. Thus, naturally resistant rabbits of Race III require as many as 1000 tubercle-bacilli units to generate a single macroscopic tubercle. On the other hand, rabbits of

TABLE 4

RATE OF MULTIPLICATION OF INHALED HUMAN-TYPE TUBERCLE BACILLI IN THE LUNGS OF GENETICALLY SUSCEPTIBLE AND RESISTANT RABBITS

Interval after infection in days	*Number of rabbit*		*Number of bacilli cultured from lungs of*		*Rate of change from bacilli estimated as inhaled*	
	susceptible	*resistant*	*susceptible*	*resistant*	*susceptible*	*resistant*
0	C7-51	IIIR 2–7	1,080[a]	1152[a]		
13	C7-51	IIIR 2–7	32,418[b]	1300[b]	×30 incr.	×1.1 incr.
33	C7-43	—	29,760,000[b]	—	×29,000 incr.	—
36	FC2-29	III 2–6	43,225[c]	0[d]	×71 incr.	Complete destruction
47	FC2-7	III 3–13	1,861,000	45	×827 incr.	×12.5 reduction
166	—	III 3–14		0	—	Complete destruction
168	—	III 3–18	—	1 colony isolated from 12 cultures[e]	—	Almost complete destruction

[a] Estimated as inhaled on basis of number of tubercle bacilli in respired air.
[b] Bacilli recovered from treated specimens of lung.
[c] From grossly unaffected lung after treatment. Inoculated guinea pig developed generalized tuberculosis.
[d] From grossly unaffected lung after treatment and guinea pig inoculation.
[e] Guinea pig inoculation—negative.

the susceptible strain, FC, require no more than 50 to generate a caseous tubercle. FIGURE 8 illustrates this observation. Furthermore, while in 90 per cent of susceptible rabbits there is a more or less extensive pulmonary disease even 5 months after exposure, there is no gross evidence of tuberculosis in 84 per cent of the resistant rabbits that live more than 2 months after the inhalation. FIGURES 9 and 10 illustrate this difference.

It has been demonstrated further, by serial culture of exposed susceptible and resistant rabbits, that the human-type tubercle bacillus grows abundantly for a long time in susceptible rabbits and generates typical caseous lesions (FIGURE 11). On the other hand, in the resistant animals, the human bacilli scarcely multiply at all, even before allergic irritability has been established, and very soon are completely destroyed. This fact is illustrated in TABLE 4. Again, in the grossly unaffected lung parenchyma of the susceptible animal, living human bacilli are present in great numbers, whereas in

the resistant animals very few remain or are completely destroyed, as revealed by culture and guinea-pig inoculation. Thus, the genetically resistant rabbits have an innate, non-specific, greater capacity to inhibit the growth of inhaled human-type tubercle bacilli than the susceptible rabbits have before any specifically acquired immune processes can be mobilized.

It is interesting to note, in this relation, that with moderate numbers of inhaled bacilli, resistant rabbits develop tuberculin sensitivity faster than susceptible rabbits. It is plain that living, non-disintegrated bacilli cannot sensitize, for they have not released their antigens. Only as the bacilli die or are destroyed, can the liberated antigens sensitize the tissues. Hence, resistant animals develop allergic sensitivity faster than susceptible animals, for they destroy the bacilli more rapidly than the susceptible animals.

The application of this knowledge to the problem of chemotherapy is evident. It is probable that susceptible rabbits, thus quantitatively exposed and treated with effective bactericidal or bacteriostatic agents, would show very few, if any, primary tubercles within 30 days after infection, by comparison with similarly susceptible, untreated rabbits simultaneously exposed to the same number of human-type virulent tubercle bacilli.

Summary

A method has been outlined for producing localized ulcerative pulmonary pthisis in vaccinated, genetically resistant rabbits, by the quantitative inhalation of small numbers of bovine tubercle bacilli. The disease thus generated closely simulates the adult or reinfection type of cavitary pulmonary tuberculosis in man. The suitability of such a disease for the study of therapeutic agents in ulcerative pthisis in man; the effect of such agents on the progress and healing of cavities; and the *in vivo* emergence of strains of tubercle bacilli resistant to the agent in question have been discussed.

A method for studying short-term therapeutic procedures in tuberculosis, by the quantitative exposure of genetically susceptible rabbits to the inhalation of human-type tubercle bacilli, has also been outlined.

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